LIGHT-INDUCTION OF SHEDDING OF GAMETES IN CIONA INTESTINALIS AND MOLGULA MANHATTENSIS[1]

D. G. WHITTINGHAM

Johns Hopkins School of Hygiene & Public Health, Baltimore, Maryland

Castle (1896) and Conklin (1905) observed that the release of gametes in three species of solitary ascidians (*Ciona intestinalis, Styela partita* and *Molgula manhattensis*) occurred at definite times during the daylight period. *Ciona intestinalis* and *Molgula manhattensis* spawn one to one and a half hours before sunrise and *Styela partita* spawns during the late afternoon. Grave (1921, 1937) found, in the colonial ascidian *Amaroucium constellatum*, that the greatest release of larvae occurred at and just before sunrise but they continued to be liberated in small numbers throughout the daylight period. He reported a similar phenomenon in *Botryllus schlosseri;* in this colonial form the larvae are released in increasingly greater numbers as the day advances, finally reaching a maximum at noon. Rose (1939) recorded that *Styela partita* could be induced to shed their eggs by subjecting them to 11–12 hours of light prior to the desired time of spawning. He observed that the natural time for shedding occurred in the laboratory between 4 and 7 PM. Furthermore, spawning under experimental conditions could be induced on four or five successive days by controlling the illumination. As yet there has been no study made of the characteristics of the illumination necessary to cause shedding.

In the present study the effects of the intensity and the wave-length of light upon shedding have been investigated in two solitary oviparous ascidians which shed at dawn—*Ciona intestinalis* and *Molgula manhattensis*. Before this was done, however, it was necessary to determine the exact times of shedding in these two species.

Materials and Method

Ciona intestinalis and *Molgula manhattensis* were obtained from the supply department at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the months of June, July and August, 1965. These animals were kept for several days in laboratory aquaria; those ascidians which were sexually mature and shedding gametes were selected for experimental purposes, and after being subjected to a particular treatment they were discarded.

All experiments were conducted in a room where the ascidians were subjected to a standard day consisting of 12 hours of light and 12 hours of darkness. In order that two series of experiments could be carried out simultaneously, animals were kept in black-painted light-tight boxes during the period of 12 hours of darkness.

[1] This work was carried out during the summer of 1965 at the Marine Biological Laboratory, Woods Hole, Massachusetts, while the author was a recipient of a Pennsylvania Plan Fellowship in the Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia. Support was made available through training grant 5T1-HD 26-04 from the National Institutes of Health.

In one experiment light was provided from 9 AM to 9 PM, while in the second experiment light was provided from 9 PM to 9 AM. In the method outlined above some of the experiments were carried out under conditions of lighting which were completely reversed from the conditions existing in the animals' natural habitat. No apparent differences were observed between animals shedding at approximately their normal time and those shedding in the reversed system of lighting.

The ascidians were kept individually in fingerbowls containing 250 ml. of filtered sea water which was changed every 12 hours. The temperature of the sea water was recorded at the time it was placed in the fingerbowl and at the end of the 12-hour period just before it was replenished. The fingerbowls were placed on a black surface to keep the reflection of light from the source to a minimum. (Reflecting power of a black painted matt surface is less than 1%.) The shedding response was recorded as either positive when gametes were released or negative when no gametes were shed.

Intensity

In the first series of light-intensity experiments, a standard light source—100-watt bulb—was used and the intensity varied by placing the ascidians at different distances from the light source. The intensity of illumination from a constant light source varies inversely as the square of the distance from that source. Theoretical values of light intensity at various distances from the light source may be calculated from the following equation:

$$\frac{\text{Intensity of illumination at A}}{\text{Intensity of illumination at B}} = \frac{d_B^2}{d_A^2}$$

where d_A and d_B are the distances of A and B from the light source, respectively, since manufacturers supply data on the intensity of illumination at 1 foot.

The shedding response was measured at 1 foot, 2 feet, 4 feet, and 8 feet from the light source and the intensity of illumination was measured with a light meter to check the theoretical calculations. Groups of six animals were placed in each treatment and their shedding responses recorded on four successive days. In these experiments the time when shedding occurred after the animals were introduced into the light was tabulated for both species of ascidians.

In a second series of light-intensity experiments four different light sources—60-watt, 40-watt, 25-watt, and 15-watt bulbs—were used at a standard distance of eight feet from the animals.

Wave-length

Four Kodak Wratten gelatin filters were used to determine the effects of the wave-length of light upon shedding. The four filters had the following characteristics: (1) Filter No. 2B absorbed light at wave-lengths of 390 mμ and below, the approximate colors absorbed were ultraviolet and violet light. (2) Filter No. 16 absorbed light at wave-lengths of 500 mμ and below, the approximate color range absorbed being blue, blue green, and those colors absorbed by filter No. 2B. (3) Filter No. 25 absorbed light at wave-lengths of 600 mμ and below, the approximate color range absorbed being green and yellow plus those colors absorbed by filter

No. 16. (4) Filter No. 89B absorbed light at wave-lengths of 700 mμ and below, the approximate color range absorbed being orange and red plus those colors absorbed by filter No. 25. Each filter measured 10 cm. by 12 cm. and it was placed in a darkroom safelight. A fixed intensity of 32 foot-candles was used; this intensity had been found to produce optimum shedding responses in previous studies on the effect of light-intensity on this process. Shedding responses were recorded on two successive days. Three separate trials were made with *Ciona intestinalis;* in each trial nine animals were allotted to a treatment. *Molgula manhattensis* was not as plentiful and 6 animals were used per treatment and 2 trials were made.

Results and Discussion

1. *Time of shedding*

In the first series of light-intensity experiments the time when shedding commenced after the ascidians were introduced into light was recorded. The data for

Table I

Mean shedding times in minutes for Ciona intestinalis recorded at 4 different intensities of light

Six animals per light-intensity, shedding responses recorded on 4 consecutive days

Day	1		2		3		4	
Light intensity (foot-candles)	Number shedding	Mean shedding time	Number shedding	Mean shedding time	Number shedding	Mean shedding time	Number shedding	Mean shedding time
130	6	3.67	6	3.17	4	2.5	4	3.75
32	6	7.17	5	5.2	5	3.6	5	6.8
8	4	3.0	4	2.25	4	3.5	5	6.0
15	2	2.0	3	4.0	5	2.6	3	2.67
Total number shedding	18		18		18		17	
Mean shedding time		4.5 ±3.22		3.67 ±1.87		3.06 ±1.11		5.12 ±3.33

Mean shedding time for 24 animals during 4-day period—4.07 min. ±2.60.

Ciona intestinalis and *Molgula manhattensis* are presented in Tables I and II, respectively. In each species 24 animals were allotted at random to the 4 light intensities so that the responses of 6 animals were observed at each light-intensity. The animals were stimulated to shed on 4 occasions, 24 hours apart. Mean shedding times at the 4 light-intensities are given for each group of 6 animals on the 4 days when shedding responses were observed. In addition, the number of animals in these groups giving positive shedding responses on each occasion is tabulated. In *Ciona intestinalis* analyses of variance showed no significant differences in the shedding time of the animals between 4 levels of light-intensity. A similar analysis of the data from *Molgula manhattensis* indicated a difference in the shedding time of the animals between intensities on the first day ($0.01 > P > 0.001$) but no differences were found on the three subsequent days. At the highest light-intensity (130 foot-candles) the mean time taken for the 6 animals to shed after exposure to light (16.2 min.) was less than the mean times for the three lower intensities (26.5 min., 23.2 min., and 22.2 min.). It seems unlikely that this is a true effect of light-

TABLE II

Mean shedding times, in minutes for Molgula manhattensis recorded at 4 different intensities of light

Six animals per light-intensity, shedding responses recorded on 4 consecutive days

Day	1		2		3		4	
Light intensity (foot-candles)	Number shedding	Mean shedding time	Number shedding	Mean shedding time	Number shedding	Mean shedding time	Number shedding	Mean shedding time
130	5	16.2	5	23.8	2	28.5	2	27.5
32	6	26.5	6	23.67	6	25.5	1	31.0
8	6	23.2	4	27.75	4	26.25	2	26.0
1.5	5	22.2	4	24.25	5	24.0	5	22.2
Total number shedding	22		19		17		10	
Mean shedding time		22.78 ±5.36		24.68 ±3.53		25.59 ±3.98		24.9 ±3.73

Mean shedding time for 24 animals during the 4-day period—24.16 min. ±4.46.

intensity upon the time of shedding since it is not repeated on the subsequent days; however, these animals may have adapted themselves to this high intensity of light on the latter three days of the experiment.

Mean shedding times for both species were calculated from the data obtained during the whole experiment. In *Ciona intestinalis* shedding occurred in 22/24 of the animals used and 74% (71/96) positive shedding responses were obtained

TABLE III

Shedding response patterns of Ciona intestinalis and Molgula manhattensis observed on 4 consecutive days

Shedding response patterns Day					*Ciona intestinalis* Light-intensities					*Molgula manhattensis* Light-intensities (Foot-candles)				
Type	1	2	3	4	130	32	8	1.5	Total	130	32	8	1.5	Total
1	1	1	1	1	4	5	3		12	1	1	1	3	6
2	1	1	1	0						1	5	2	1	9
3	1	1	0	1						1				1
4	1	1	0	0	2				2	1		1		2
5	1	0	1	1			1	1	2			1	1	2
6	1	0	1	0				1	1					
7	1	0	0	1										
8	1	0	0	0		1			1	1		1		2
9	0	1	1	1				2	2					
10	0	1	1	0				1	1					
11	0	1	0	1			1		1					
12	0	1	0	0						1				1
13	0	0	1	1										
14	0	0	1	0										
15	0	0	0	1										
16	0	0	0	0			1	1	2				1	1

1 = shedding
0 = no shedding

during the 4 days. This species commenced shedding gametes 4.07 min. ± 2.60 after exposure to light. In *Molgula manhattensis* 23/24 animals shed and 71% (68/96) positive shedding responses were obtained during the 4 days; the overall mean time when shedding commenced was 24.16 min. ± 4.46 after exposure to light. Therefore, from the data above it has been shown that these two species of ascidians have their own characteristic shedding time. If the light stimulus mediates its effect *via* the neural ganglion and neural gland complex of these animals, the response of these structures to the light-stimulus occurs much more rapidly in *Ciona intestinalis* than in *Molgula manhattensis*.

Since the behavior of each animal was observed on 4 successive days its overall response pattern can be represented by a vector of 4 elements, 1 and 0 representing shedding and not shedding, respectively. There are thus 16 possible response patterns. The distribution of the shedding response patterns of the two species observed on the four consecutive days is listed in Table III. Fifty per cent (12/24)

TABLE IV

Percentage of Ciona intestinalis and Molgula manhattensis shedding in response to different light-intensities

Light intensities (foot-candles)	Number of animals	% Shedding	
		Ciona intestinalis	*Molgula manhattensis*
130	6	100.00	83.33
32	6	83.33	83.33
8	6	66.67	66.67
1.5	6	50.00	66.67
1.17	12	41.67	58.33
0.67	12	41.67	66.67
0.40	12	16.67	58.33
0.21	12	0	25.00

of *Ciona intestinalis* shed on four days whereas only 25% of *Molgula manhattensis* shed consecutively over a similar period. The highest shedding responses occurred on the first two days of the experiment; the fall in response over the last two days may have been due to the lack of food materials or to the handling of the animals.

2. *Light-intensity*

The shedding responses of the two ascidians to 8 different light-intensities are presented in Table IV. These responses were recorded on the second day of exposure to the various light-intensities. The high intensities of light did not inhibit the shedding response. In *Ciona intestinalis* shedding was reduced to 16.67% at 0.40 foot candle and was completely inhibited at 0.21 foot-candle. The response in *Molgula manhattensis* was reduced to 25% at the latter light-intensity. The active contractions of the animals associated with the light-stimulus (Hecht, 1926) and the shedding of gametes (Castle, 1896) were diminished in both species at the lowest light-intensity (0.21 foot-candle). It may be suggested that the release of gametes is a reflex associated with the muscular contractions of the animal stimulated by exposure to light.

In the present study, it has been shown that these species shed at low as well as high light-intensities which would allow them to adapt to a fairly wide range of naturally occurring habitats (Van Name, 1945).

3. *Wave-length*

The data obtained showing the shedding response of the two species when the wave-lengths of light were restricted to certain regions of the spectrum are presented in Table V. In both species the shedding response was reduced to below 50% when light was absorbed up to 600 mμ and totally inhibited when absorbed up to 700 mμ. These results indicate that the excitatory wave-lengths occur between 500 mμ and 700 mμ. A constant light-intensity was used in these experiments—32 foot-candles—and therefore no interaction between light-intensity and wave-length was shown; however, further investigation of this aspect may help in the understanding of the shedding phenomenon. In the experiments described, the

TABLE V

Percentage of Ciona intestinalis and Molgula manhattensis shedding in response to restricted wave-lengths of light

Wave-length absorption	*Ciona intestinalis*		*Molgula manhattensis*	
	Number of animals	% Shedding	Number of animals	% Shedding
390 mμ and below	27	81.84	12	91.67
500 mμ and below	27	62.96	12	83.33
600 mμ and below	27	18.52	12	33.33
700 mμ and below	27	0	12	0

wave-length range which stimulates shedding has good transmission through sea water (Jerlov, 1964) and these wave-lengths would reach the animals in their natural habitat.

The author gratefully acknowledges the advice and guidance given to him by Dr. J. D. Biggers during the course of the work and in the preparation of the manuscript.

SUMMARY

1. The time of shedding of gametes and the effects of the intensity and the wave-length of light upon the process have been investigated in *Ciona intestinalis* and *Molgula manhattensis*.

2. The time of shedding was recorded from 24 animals in each species on four consecutive days. It was found that *Ciona intestinalis* and *Molgula manhattensis* commenced to shed their gametes 4.07 min. ± 2.60 and 24.16 min. ± 4.46, respectively, after exposure to light. Twelve out of 24 *Ciona intestinalis* shed on 4 consecutive days and 6 out of 24 *Molgula manhattensis* shed consecutively over a similar period.

3. Intensities ranging from 0.21 to 130 foot-candles (f.c.) were used to study light-intensity effects. High intensities did not inhibit shedding but lower intensities —0.67 f.c. and 0.40 f.c.—produced a marked reduction in the shedding response. At 0.21 f.c. shedding was completely inhibited in *Ciona intestinalis* and reduced to 25% in *Molgula manhattensis*.

4. In both species, shedding was partially inhibited when light was absorbed up to 600 mμ and totally inhibited when absorbed to 700 mμ. This indicated that the excitatory wave-lengths lie between 500 mμ to 700 mμ.

LITERATURE CITED

Castle, W. E., 1896. The early embryology of *Ciona intestinalis* (L). *Bull. Mus. Comp. Zool.*, **27**: 201–280.

Conklin, E. G., 1905. Organization and cell lineage of the ascidian egg. *J. Acad. Nat. Sci., Philadelphia*, **13**: 1–21.

Grave, C., 1921. *Amaroucium constellatum* (Verrill) II. The structure and organization of the tadpole larva. *J. Morph.*, **36**: 71–91.

Grave, C., 1937. *In*: Culture Methods for Invertebrate Animals. Ed. Galtsoff *et al.*, Comstock, Ithaca, pp. 556–564.

Hecht, S., 1926. The effect of exposure period and temperature on the photosensory process in *Ciona*. *J. Gen. Physiol.*, **8**: 291–301.

Jerlov, N. G., 1964. Colour filters to simulate the extinction of daylight in the sea. *Extrait du Journal du Conseil International Pour l'Exploration de la mer*, **20**: 156–159.

Rose, S. M., 1939. Embryonic induction in the ascidians. *Biol. Bull.*, **77**: 216–232.

Van Name, W. E., 1945. The North and South American ascidians. *Bull. Amer. Mus. Nat. Hist.*, **84**: 1–476.